Lasers in Surgery and Medicine 21:493–499 (1997)

Changes in Calcium Transport in Mammalian Sperm Mitochondria and Plasma Membranes Caused by 780 nm Irradiation

Rachel Lubart, PhD,[1] Harry Friedmann, PhD,[1] Michael Sinyakov, PhD,[2] Natalie Cohen, MSc,[2] and Haim Breitbart, PhD[2]*

[1]*Department of Physics and Chemistry, Bar-Ilan University, Ramat Gan, Israel*
[2]*Department of Life Sciences, Bar-Ilan University, Ramat Gan, Israel*

Background and Objective: **Regulation of intracellular Ca^{2+} concentrations are very important in control of sperm motility and acrosome reaction. It was shown previously that low-power lasers in the visible and near-infrared range alter Ca^{2+} uptake by sperm cells. In the present work the effect of a 780 nm diode laser on Ca^{2+} uptake by sperm mitochondria and isolated plasma membrane vesicles is investigated.**
Study Design/Materials and Methods: **Digitonin-treated spermatozoa and plasma membrane vesicles were irradiated with a 780-nm diode laser at various powers and energy doses, and Ca^{2+} uptake was measured by the filtration method.**
Results: **It was found that 780-nm irradiation inhibits Ca^{2+} uptake by the mitochondria but stimulates Ca^{2+} binding by sperm plasma membrane vesicles. The effect of light on Ca^{2+} uptake by plasma membrane vesicles in the absence of ATP was much larger than that measured in the presence of ATP. Addition of Ca^{2+} ionophore decreased the Ca^{2+} uptake by the irradiated membranes in the presence of ATP but enhanced it significantly in the absence of ATP.**
Conclusion: **780 nm light inhibits Ca^{2+} uptake by sperm mitochondria and enhances Ca^{2+} binding to sperm plasma membranes. Lasers Surg. Med. 21:493–499, 1997.**

Key words: Ca^{2+} binding; spermatozoa; plasma membrane vesicles; 780 nm diode laser

INTRODUCTION

Intracellular calcium plays a vital role in cell proliferation, and in mammalian spermatozoa it has a pivotal role in control of sperm motility [1] and acrosome reaction [2]. Therefore, the alteration in calcium levels in response to exposure to light may have considerable biological and clinical significance.

It was found that irradiating various cells with certain wavelengths at specified energy doses in the visible and in the far-red range resulted in acceleration of the Ca^{2+} uptake by the cells [3,4].

Light in the visible and far-red range can be absorbed by mitochondrial enzymes [5]. Therefore, it was suggested [6,7] that HeNe laser radiation activates the redox reactions in the respiratory chain by exciting mitochondrial porphyrins or cytochromes. This activation could lead to changes in $[Ca^{2+}]_i$ in the cell. We have fixed our attention on the effect of light on Ca^{2+} transport in sperm cells. The systems which regulate intracellular Ca^{2+} concentration in spermatozoa in-

*Correspondence to: Haim Breitbart, Department of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel. E-mail: breith@ashur.cc.biu.ac.il

Accepted 24 July 1997

volve the mitochondria, [8,9], the plasma membrane ATP-dependent Ca^{2+} pump [10,11], the Na^{+}/Ca^{2+} antiport [12,13], and the Ca^{2+} channel [14]. In a previous work [3], we measured Ca^{2+} uptake by sperm mitochondria irradiated with HeNe (633 nm) light and found acceleration or inhibition of Ca^{2+} uptake, depending on the energy doses of the light. Irradiation of isolated plasma membrane vesicles with HeNe laser did not change their Ca^{2+} uptake ability.

In the present work we have examined Ca^{2+} uptake by sperm mitochondria and isolated plasma membrane vesicles irradiated with 780 nm light. At powers and energy doses used in our experiments, we found that Ca^{2+} uptake was inhibited in the mitochondria and increased in the plasma membrane vesicles. In the case of isolated plasma membrane vesicles we have investigated the nature of this Ca^{2+} uptake in the presence and absence of ATP and Ca^{2+}-specific ionophore A23187. We found that the elevation in Ca^{2+} uptake by the plasma membrane was a consequence of Ca^{2+} binding to the membrane after irradiation.

MATERIALS AND METHODS

Frozen ejaculated bull sperm cells (from Hasherut Artificial Insemination Center) were thawed at 37°C in medium comprising 150 mM NaCl, 10 mM histidine (pH 7.4). The cells were washed by three centrifugations at 600*g*, at 25°C for 10 min. The washed cells were resuspended in buffer containing 110 mM NaCl, 5 mM KCl, and 10 mM sodium morpholinopropanesulfonate (MOPS), pH 7.4.

Irradiation

A schematic representation of the irradiation set-up is given in Figure 1. The light source was a 780 ± 5-nm, 25-mW diode laser (Lasotronics). An optical multimode fiber made of silica glass was connected to the laser and was inserted into a rotating tube, at 37°C, containing 2–5 ml cell suspension of 3×10^8 cells/ml or 30 μg prot./ml of membranes. The light power at the end of the fiber ranged from 3 mW to 25 mW. (Neutral density filters were used to reduce the intensity of the laser.) The irradiation time was 1–20 minutes. All light intensities were measured with an Ophir (model PD2-A) power meter.

The experimental set-up in this work differs from that used in our previous experiments on intact spermatozoa [3]. With the intact spermatozoa the Ca^{2+} uptake was measured while the cells were transferred to 96-well culture plate at room temperature and the light was defocused on each well. As we preferred to measure Ca^{2+} uptake into plasma membrane vesicles at 37°C and not at room temperature, we had to transfer the light through a fiber into a rotating tube immersed in a 37°C water bath [15]. In such a set-up, time of exposure of the cells to the light beam is unknown, so the results can only be qualitatively compared with those obtained previously [3] on intact sperm cells.

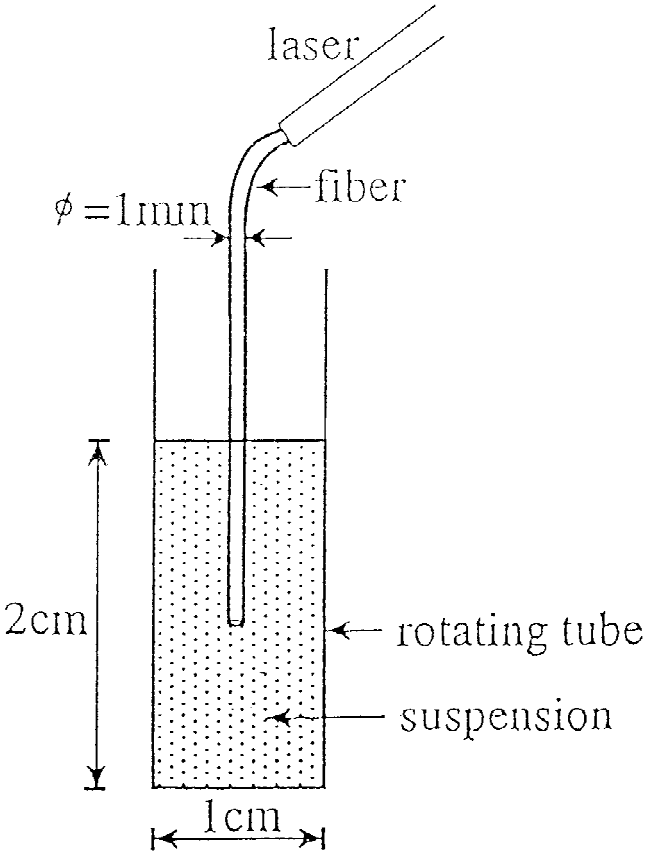

Fig. 1. A schematic representation of the irradiated sample.

Digitonin treatment. Since it is impossible to isolate coupled mitochondria from spermatozoa, we have decided to follow calcium transport into permeabilized sperm. Under these conditions, the Ca^{2+} uptake is completely abolished by the mitochondrial uncoupler CCCP [2,7] (carbonyl cyanide para trifluoro methoxy phenyl hydrazone), indicating that the transported Ca^{2+} is accumulated in the mitochondria. Thus, we could test the irradiation effect on mitochondrial Ca^{2+} transport. A suspension of 4×10^8 cells/ml in 5-ml buffer A was mixed with 65.5 μl of 14.27 mM dig-

itonin in dimethylsulfoxide. When motility was completely stopped, the suspension was centrifuged at 4°C at 600*g*. The pellet was resuspended with buffer M composed of 250 mM mannitol, 70 mM sucrose, and 10 mM TEA-Hepes (pH = 7.4), centrifuged as above, and then suspended in cold buffer M and kept on ice.

Preparation of sperm plasma membranes. The sperm plasma membranes were prepared as previously described by us [10,11]. The sperm cells were pelleted by centrifugation at 1,500*g* for 10 min; then the cells were washed four times in buffer NKM (110 mM NaCl, 5 mM KCl, and 10 mM MOPS, pH 7.4). The washed cells were resuspended in hypotonic medium (10 mM histidine (pH 7.4)/0.5 mM EDTA) and disrupted by using the Ultraturrax homogenizer (Janke and Kunkel K6 IKA WERK Typ. TP18-10) in the following way: 10 s low rate, 3 s high, 7 s low, 3 s high, and 7 s low rate. Low and high rates represent 3,000 and 14,000 rpm, respectively. The suspension was centrifuged at 3,000*g* for 10 min, and the supernatant was removed and centrifuged at 6,000*g* for 10 min. The supernatant was removed and centrifuged at 35,000*g* for 30 min; then the pellet was resuspended in the hypotonic medium. The suspension was layered on a discontinuous sucrose gradient composed of 0.5, 1.0, and 1.5 M sucrose solutions prepared in 10 mM histidine (pH 7.4). The tubes were centrifuged at 30,000 rpm for 18 h at 4°C using a SW 41 rotor, in Spinco (Beckman) ultracentrifuge. The membrane fraction located just above the 1.5 M sucrose layer was removed and diluted with 10 mM histidine (pH 7.4)/0.1 mM EDTA at 4°C. The protein concentration was determined by the method of Lowry et al. [16] using bovine serum albumin as the standard of reference. The membranes were stored at −20°C prior to analysis. These membranes showed a 45-fold enrichment of the plasma membrane marker ($Na^+ + K^+$) ATPase and less than 4% of the mitochondrial marker cytochrome c oxidase–specific activity found in whole cell homogenates. When examined by transmission electron microscopy, the membranes were vesicular, and mitochondria were not identified [see ref. 10,11].

Calcium uptake by permeabilized cells. Uptake of $^{45}Ca^{2+}$ was determined by the filtration technique. Cells (3×10^8 ml) were incubated in a medium containing 10 mM lactate, 0.5 mM phosphate, 0.2 mM $CaCl_2$, and 0.5 μCi $^{45}CaCl_2$. The cells were irradiated with 780-nm light (4 mW, 9 mW, 24 mW) immediately after the addition of calcium. At various time intervals (up to 12 min) 0.1 ml was removed and immediately vacuum-filtered on GF/C filters. The cells trapped on the filter were washed three times with 5 ml of solution composed of 150 mM NaCl, 10 mM Tris (pH 7.4), and 2 mM EGTA. The dry filters were counted in scintillation vials with 5 ml Aquasol (DuPont). Calcium uptake was calculated as nmol $Ca^{2+}/10^8$ cells.

Calcium uptake by isolated plasma membranes. Ca^{2+} uptake by spermatozoa plasma membrane vesicles was measured in a 0.8 ml medium containing 18 mM histidine/18 mM imidazole buffer (pH 6.8), 0 1M KCl, 3 mM $MgCl_2$, 0.18 mM $CaCl_2$ (10 μM free Ca), 1 μCi $^{45}CaCl_2$, 0.2 mM EGTA, and 240 μg plasma membrane protein. In some experiments, 10 mM A23187 Ca-specific ionophore (CalBiochem) was added. After a 5-min preincubation at 37°C, Na-ATP was added to achieve a final concentration of 2 mM ATP. Part of the experiments were done without exogenous ATP. The reaction mixture was incubated at 37°C and irradiated with 780 nm light. At appropriate time intervals, samples (100 μl) were removed and vacuum filtered on 0.45-μm millipore filters. The membrane vesicles trapped on the filter were washed with cold water and placed in scintillation vials for measurement of the β radioactivity.

The experiments were carried out in triplicate for a single preparation of ejaculated sperm and at least three different preparations, each taken from a different animal, were used. The percentage of Ca^{2+} uptake of each sample relative to the maximum Ca^{2+} uptake of a non-irradiated sample in the absence of ATP was calculated. The results of at least three preparations were analyzed statistically by Student's t-test and analysis of variance.

RESULTS

Exposure of sperm mitochondria (permeabilized cells) to 780-nm light at 4 mW and 9 mW at various energy doses, 0.2–6.0 J, resulted in Ca^{2+} uptake inhibition (Fig. 2). We used permeabilized sperm cells because it is difficult to isolate coupled sperm mitochondria. Their separation requires the breakage of disulfide bridges [17]. Digitonin treatment disrupts the sperm plasma membrane while leaving the mitochondria functionally intact [18]. When digitonin-treated cells were washed free of phosphate and mitochondrial substrate, little Ca^{2+} uptake occurred. However, a

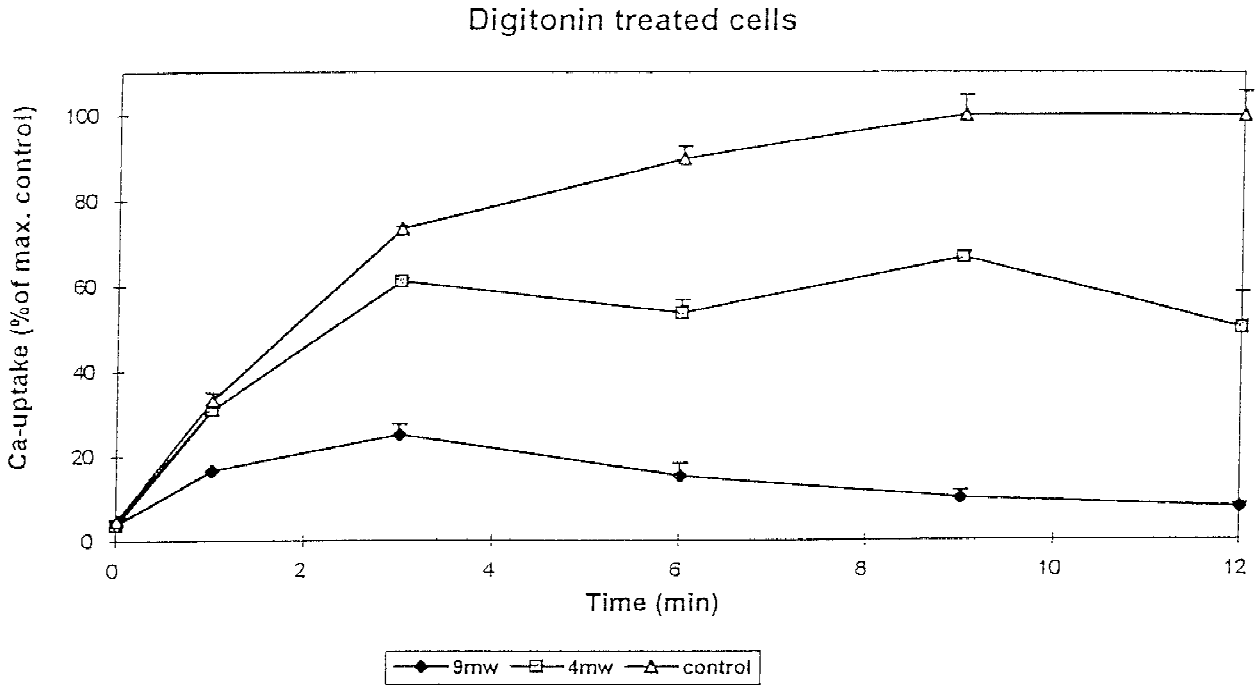

Fig. 2. Ca^{2+} uptake by irradiated digitonin-treated cells at 780 nm, relative to maximum Ca^{2+} uptake by non-irradiated digitonin-treated sperm cells. The time in the graph represents time of irradiation and time of Ca^{2+} uptake. The values are the mean ± S.D of three experiments each performed in triplicate. The difference between the control and the light-treated cells is highly significant ($P < .05$ for the 4-mW diode laser and $P < .01$ for the 9-mW laser). The 100% represents Ca^{2+} uptake by control cells after 12 min of incubation. (100% = 14.05 nmol $Ca^{2+}/10^8$ cells). The Ca^{2+} uptake oscillation in response to 4-mW irradiation does not always appear in samples from different bulls.

high degree of Ca^{2+} uptake can be observed with the addition of mitochondrial substrate and phosphate. In this work we used lactate as a sperm mitochondrial substrate. Ca^{2+} uptake into permeabilized cells is above 90% inhibited by mitochondrial uncoupler (data not shown), indicating that most of the absorbed Ca^{2+} is accumulated in the mitochondria.

Irradiation of plasma membrane vesicles with 25 mW 780-nm light at energy doses of 1.5–30 J enhanced Ca^{2+} uptake by the membranes (Fig. 3). A similar enhancement was achieved with a 9-mW 780-nm laser (data not shown). Comparison of the relevant pairs, namely irradiated versus non-irradiated membrane specimens, reveals clear-cut statistically significant differences and indicates that the effect of light in the absence of ATP ($P < .01$) is much larger than that observed in the presence of ATP ($P < .05$). Ca^{2+} uptake in the non-irradiated control specimens was significantly higher in the presence of ATP as compared to that in the absence of ATP ($P < .003$), while the difference in Ca^{2+} uptake in the irradiated samples was not significant.

Addition of Ca^{2+}-specific ionophore A23187 to the reaction mixture in the absence of ATP (Fig. 4) resulted in a very small decrease of Ca^{2+} uptake in the non-irradiated control membrane preparations ($P < .003$). At the same time, the ionophore did not eliminate the effect of 780-nm irradiation; moreover, Ca^{2+} uptake in the irradiated membranes was additionally and significantly ($P < .002$) increased in the presence of the ionophore.

In the presence of ATP, addition of the ionophore (Fig. 5) drastically and significantly decreased Ca^{2+} uptake in both the non-irradiated control membranes ($P < .003$) and the irradiated specimens ($P < .03$); in the latter case, Ca^{2+} uptake in the presence of ionophore dropped to the level of the non-irradiated control in the absence of ionophore (see Fig. 5). The effect of light on Ca^{2+} uptake was much higher (see Figs. 4, 5) in the presence of the Ca^{2+}-ionophore than in its absence. In Figure 5, the light effect in the presence of A23187 seems to be lower in comparison to laser alone. But, if we subtract the controls, we find 6 times enhancement of Ca^{2+} uptake by light in the presence of A23187, and only 1.6-fold increase in its absence.

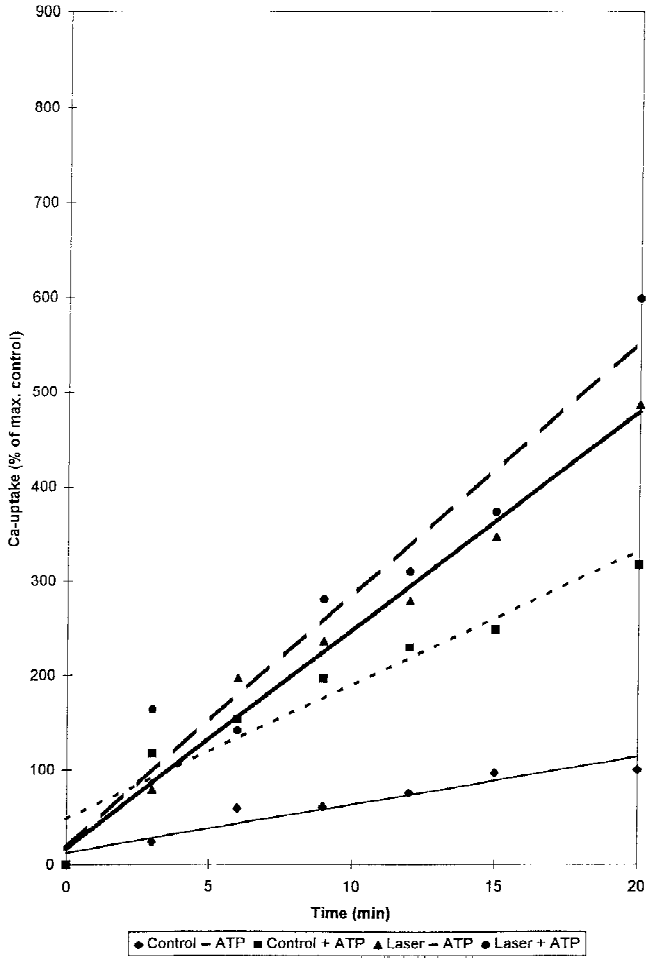

Fig. 3. Ca^{2+} uptake by irradiated (25 mW, 780 nm) plasma membrane vesicles from sperm cells in the presence or absence of ATP. The 100% represent Ca^{2+} uptake by non-irradiated membranes in the absence of ATP at 20 min uptake time and corresponds to the uptake of 1.48 nM Ca^{2+}/mg membrane protein. The time in the graph represents time of irradiation and time of Ca^{2+} uptake. Each value represents the mean of three experiments performed in triplicate. In Figures 3–5 it was possible to perform a linear fit transformation of the results obtained because of the high values of the correlation coefficients which ranged from 0.88 to 0.98.

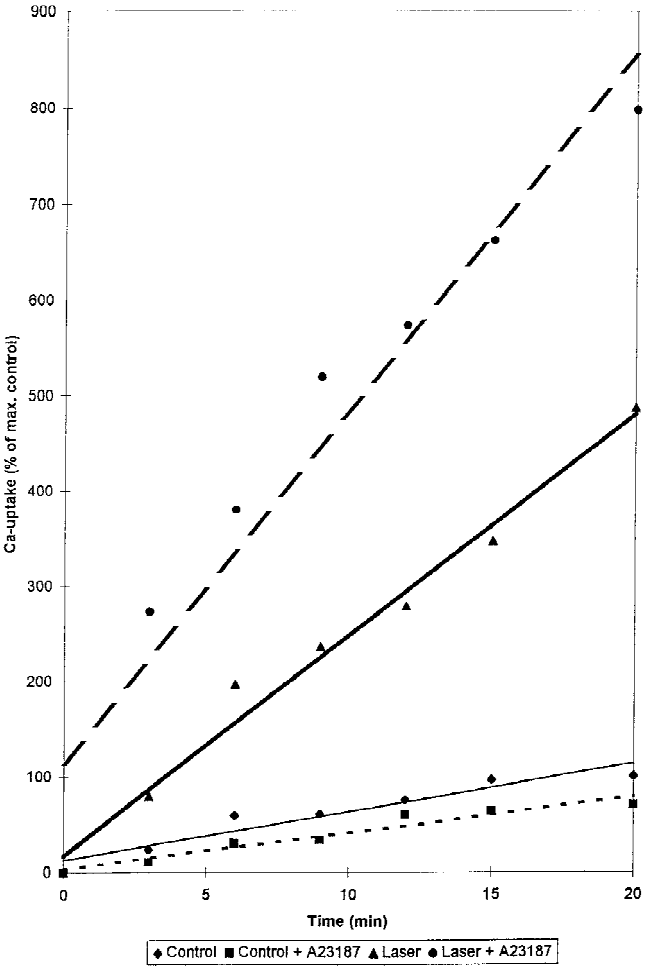

Fig. 4. Ca^{2+} uptake by irradiated (25 mW, 780 nm) plasma membrane vesicles from sperm cells ± Ca^{2+} ionophore A23187, in the absence of ATP relative to maximum Ca^{2+} uptake by non-irradiated membranes (as designated in legend to Fig. 3).

DISCUSSION

The mechanism by which light in the visible and far-red range interacts with the living cell is still being debated. As intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ is responsible for various cell activities, several authors investigated the effect of light on $[Ca^{2+}]_i$. Young et al. [4] found an increased $^{45}Ca^{2+}$ uptake by macrophages after 660-, 820-, and 870-nm irradiation. Karu [19] found an elevation of $[Ca^{2+}]_i$ in lymphocytes after HeNe irradiation. We measured changes in Ca^{2+} uptake by bovine sperm cells due to 633-nm and 780-nm irradiation [3]. We found that light in the visible and in the far-red range at a specified energy dose accelerated Ca^{2+} uptake by the various cells. In order to better understand the mechanism by which light affects the cell, we measured Ca^{2+} uptake by HeNe-irradiated sperm mitochondria (permeabilized cells) and by isolated plasma membranes [15]. We found that HeNe laser irradiation at low power (0.3 mW) and low energy doses (0.06–0.2 J), produced enhanced Ca^{2+} uptake, whereas at higher power (10 mW) and doses (0.6–7.2 J) the uptake was inhibited. No effect was observed when sperm plasma membrane vesicles were irradiated with HeNe laser.

In the present work the influence of far-red, 780-nm, laser diode radiation was examined. As described in Figure 2, 780-nm light at 0.2–6.0 J,

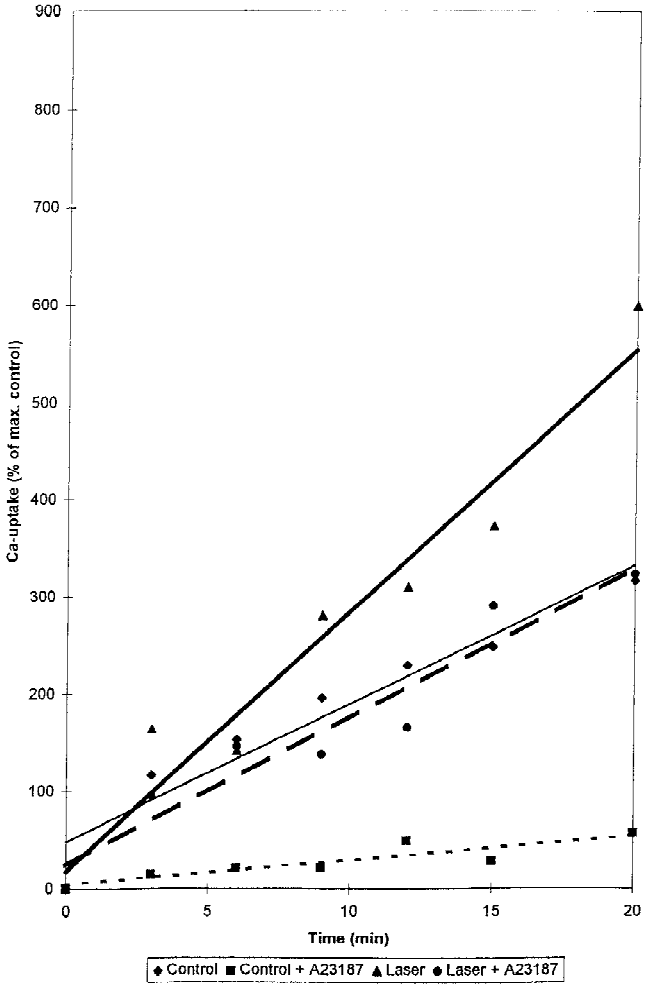

Fig. 5. Ca^{2+} uptake by irradiated (25 mW, 780 nm) plasma membrane vesicles from sperm cells in the presence of ATP and in the absence and presence of the Ca^{2+}-ionophore A23187, relative to maximum Ca^{2+} uptake by non-irradiated membranes (as designated in legend to Fig. 3).

inhibits Ca^{2+} uptake by the mitochondria. On the other hand, 780-nm light at 1.5–30 J enhanced Ca^{2+} uptake by isolated plasma membrane vesicles (Fig. 3). The cell plasma membranes regulates intracellular Ca^{2+} concentration mainly by the ATP-dependent Ca^{2+} pump [10,11], the Na^{+}/Ca^{2+} exchanger [12,13], and by a voltage-gated Ca^{2+} channel [14]. Isolated plasma membrane vesicles can accumulate a significant amount of Ca^{2+} in the presence of added ATP. Without ATP, the uptake of Ca^{2+} is very low (Fig. 3). 780 nm light stimulates the Ca^{2+} uptake, and this effect is much higher in the absence of ATP. Also, the light effect is not canceled by adding the Ca^{2+} ionophore A23187 ($Ca^{2+}/2H^{+}$ exchanger), although the ATP-dependent Ca^{2+} accumulation was completely dissipated under these conditions (Fig. 4). This indicates that 780-nm light enhances Ca^{2+} binding to the membrane and not Ca^{2+} accumulation in the intravesicular space. The fact that A23187 stimulates the light effect on Ca^{2+} uptake indicates that Ca^{2+} binding to intravesicular sites is also enhanced by the light. The observed difference in Ca^{2+} uptake by the mitochondria and the plasma membranes under HeNe (633-nm) irradiation and 780-nm diode laser irradiation indicates the importance of the effect of specific wavelength on calcium transport in sperm cells.

The stimulating action of visible and far-red light is assumed to be a consequence of light absorption by endogenous photosensitizers in the respiratory chain (RC) [6,19]. The electronically excited photosensitizers, cytochromes, or porphyrins produce reactive oxygen species (ROS) [20–22] which act as potent oxidizers stimulating the redox activity of the RC, enhancing the membrane potential ($\Delta\psi$) across the inner membrane of the mitochondria and ATP production [23]. The enhanced $\Delta\psi$ and ATP production can modulate $[Ca^{2+}]_i$ by enhancing Ca^{2+} influx into the mitochondria and by increasing the activity of the ATPase-driven pumps in the cell membrane [3,19]. However, in light of the results reported here, another possible mechanism should be considered, i.e., proteins may be the direct target of low-energy laser irradiation [24,25]. We assume that in the case of far-red light vibrational overtone excitation occurs, and not non-pigmented proteins can undergo a conformational transformation to either more activated conformation or to an inhibitory one. Thus, we ascribe the inhibition of Ca^{2+} uptake into the mitochondria irradiated by 780-nm light to inhibition of the enzymes of the RC [26] or the Ca^{2+} uniporter. On the other hand, the 780-nm radiation affects the conformation of proteins in the isolated plasma membrane in a way which increases the accessibility of Ca^{2+} to its binding sites. Alteration in Ca^{2+} binding undoubtedly can change intracellular Ca^{2+} concentration [27], leading to stimulatory effects. At high energy doses it can create the possibility of closing divalent ion channels, thus leading to cell death.

Since the effect of light is much smaller in the presence of ATP (Fig. 3), we assume that bound ATP might protect certain membranal proteins from undergoing light-induced conformational transformation. It is well known that binding of a substrate to an enzyme active site pro-

tects the enzyme from conformational transformations.

ACKNOWLEDGMENTS

We would like to thank the Israeli Ministry of Health for their support of this research and Lasotronic for providing us with the Med-140 780-nm diode laser. Many thanks to Avrille Goldreich for editing this manuscript.

REFERENCES

1. Singh JP, Babcock DF, Lardy HA. Motility activation, respiratory stimulation and alterations of Ca^{2+} transport in bovine sperm treated with amine local anesthetics and Ca^{2+} transport antagonists. Arch Biochem Biophys 1983; 221:291–303.
2. Yanagimachi R, Usui N. Calcium dependence of the acrosome reaction and activation of the guinea pig spermatozoa. Exp Cell Res 1974; 89:161–174.
3. Lubart R, Friedmann H, Levinshal T, Lavie R, Breitbart H. Effect of light on calcium transport in bull sperm cells. J Photochem Photobiol B Biol 1992; 15:337–341.
4. Young SR, Dyson M, Bolton P. Effect of light on calcium uptake by macrophages. Laser Ther 1990; 2:53–57.
5. Tamura M. Non-invasive monitoring of the redox state of cytochrome oxidase in living tissue using near infra-red laser lights. JPM Cinc J 1993; 57:817–824.
6. Friedmann H, Lubart R, Laulicht I. A possible explanation of laser-induced stimulation. J Photochem Photobiol B 1991; 11:87–95.
7. Karu T. Molecular mechanism of therapeutic effect of low intensity laser irradiation. Lasers Life Sci 1988; 2:53–74.
8. Babcock DF, First NL, Lardy HA. Action of ionophore A23187 at the cellular level. Separation of effects at the plasma and mitochondrial membranes. J Biol Chem 1976; 251:3881–3886.
9. Lehninger AL. Mitochondria and calcium ion transport. Biochem J 1970; 118:129–138.
10. Breitbart H, Stern B, Rubinstein S. Calcium transport and Ca^{2+}-ATPase activity in ram spermatozoa plasma membrane vesicles. Biochim Biophys Acta 1983; 728: 349–355.
11. Breitbart H, Rubinstein S. Calcium transport by bull spermatozoa plasma membranes. Biochim Biophys Acta 1983; 732:464–468.
12. Breitbart H, Cragoe EJ, Jr, Lardy HA. Stimulation of Ca^{2+}-uptake into epididymal bull spermatozoa by analogues of amiloride. Eur J Biochem 1990; 192:529–535.
13. Rufo GA, Schoff PK, Lardy HA. Regulation of calcium content in bovine spermatozoa. J Biol Chem 1984; 259: 2547–2552.
14. Babcock DF, Pfeiffer DR. Independent elevation of cytosolic Ca^{2+} and pH of mammalian sperm by voltage dependent and pH sensitive mechanisms. J Biol Chem 1987; 262:15041–15047.
15. Breitbart H, Levinshal T, Cohen N, Friedmann H, Lubart R. Changes in calcium transport in mammalian sperm mitochondria and plasma membrane irradiated at 633 nm (HeNe laser). J Photochem Photobiol B 1996; 34:117–121.
16. Lowry OH, Rosenbrough NH, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951; 193:265–275.
17. Bartoov B, Messer GY. Isolation of mitochondria from ejaculated ram sperm. J Ultrastruct Res 1976; 57:68–76.
18. Morton BE, Lardy HA. Cellular oxidative phosphorylation: I. Measurement in intact spermatozoa and other cells. Biochemistry 1967; 6:57–63.
19. Karu T. Depression of the genome after irradiation of human lymphocytes with He-Ne laser. Laser Ther 1992; 4:5–24.
20. Basu-Modak S, Tyrrel RM. Singlet oxygen: a primary effector in the ultraviolet A near-visible light induction of the human heme oxygenase gene. Cancer Res 1993; 53: 4505–4510.
21. Grossman N, Schneid H, Reuveni H, Halevy S, Lubart R. Enhanced proliferation of keratinocyte cultures following low power HeNe (633 nm) laser irradiation. Involvement of reactive oxygen species. J Photochem Photobiol B 1996: (in press).
22. Jung J, Kim HS. The chromophores as endogenous sensitizers involved in the photogeneration of singlet oxygen in spinach thylakoids. Photochem Photobiol 1990; 52: 1003–1009.
23. Passarella S, Casamassima E, Molinari S, Pastone D. Increase of proton electrochemical potential and ATP synthesis in rat liver mitochondria irradiated *in vitro* by HeNe laser. FEBS Lett 1984; 175:95–99.
24. Ostuni A, Passarella S, Quagliariello E. Photomodulation of glutamate dehydrogenase properties by red light. J Photochem Photobiol B 1993; 20:101–111.
25. Vacca RA, Marra E, Quagliariello E, Greco M. Increase of both transcription and translation activities following separate irradiation of the *in vitro* system components with He-Ne laser. Biochem Biophys Res Commun 1994; 203:991–997.
26. Bolognani L, Volpi N. Low power laser-enzyme interaction. In: Galleti G, Bolognani L, Ussia G, eds. "Proc. 3rd World Congress of the International Society for Low Power Laser Application in Medicine." Bologna: Monduzzi, 1992, pp 213–222.
27. Breitbart H, Wehbie RS, Lardy HA. Calcium transport in bovine sperm mitochondria: Effect of substrates and phosphate. Biochim Biophys Acta 1990; 1026:57–63.